

Enabling genome discovery in sheep: Superior DNA collection from nasal samples using Performagene™

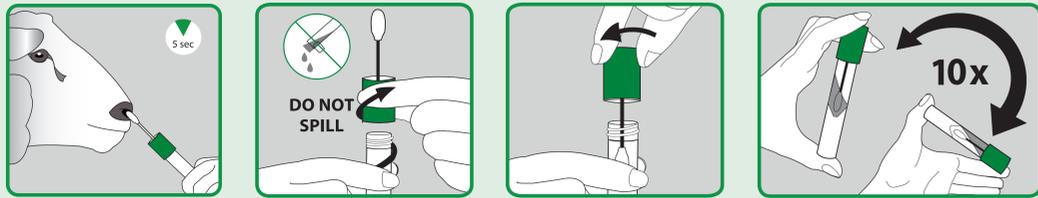
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Introduction

Genome discovery in sheep has been vastly accelerated by the introduction of the Illumina OvineSNP50 BeadChip. A reliable DNA sample must be collected in order to facilitate this genetic analysis. In order to enable large sets of DNA samples to be collected, an easy-to-use collection method is preferred. To date the source of DNA for parentage and genotyping on sheep has been blood, semen or ear tissue. These samples can be costly and inconvenient for the producer to obtain and are often difficult and inefficient to process in the lab. The reliable collection of genomic DNA from sheep nasal samples is demonstrated using the Performagene™ DNA collection kit developed by DNA Genotek, Inc. Nasal sample collection is an easy-to-use and reliable alternative that provides high quantity, high quality genomic DNA from sheep. Our study samples were collected from the nostrils of 18 young lambs and adult ewes. Paired blood samples were collected from 6 of the animals for SNP concordance analysis. Fluorescent quantification is used to demonstrate that the Performagene DNA collection method yields a median 8.2 µg of DNA per sample and median concentration of 82.2 ng/µL compared with blood samples which yield a median concentration of 25.8 ng/µL. Agarose gel analysis of sheep DNA isolated from nasal and blood samples demonstrates that high molecular weight genomic DNA is consistently obtained. Analysis from genotyping shows the suitability of nasal samples on the OvineSNP50 BeadChip with median call rates of 99.8% and genotype concordance for paired nasal and blood samples was 100%.

Materials and methods

Figure 1: Nasal sample collection using Performagene



- 18 nasal samples were collected according to the DNA Genotek protocol PD-PR-099 from 18 different sheep.
- Blood was drawn from 6 of the sheep into a standard EDTA blood tube.
- Nasal samples were shipped at ambient temperature by standard mail to DNA Genotek for processing.
- Blood samples were shipped on ice using standard precautions to DNA Genotek for processing.
- A 0.5 mL aliquot of the nasal samples was purified using PG-L2P followed by alcohol precipitation according to the DNA Genotek purification protocol PD-PR-083.
- Four 200 µL aliquots of blood were purified using the Qiagen Blood Mini Kit. The four elutions were pooled and reprecipitated.
- All of the purified DNA was quantified using Quant-It™ PicoGreen® and shipped to DNA LandMarks for QC check and analysis on the OvineSNP50 BeadChip.
- A visual QC was performed on the DNA samples and a high molecular weight test was conducted. All samples were processed on the OvineSNP50 BeadChip.

Results

Table 1: Quantification results for nasal and blood samples

Animal ID	Nasal – DNA concentration (ng/µL)	Blood – DNA Concentration (ng/µL)
SA6	43.5	29.5
SA34	47.9	40.0
SA36	99.8	
SA38	100.8	
SA39	96.0	
SA42	70.6	
SA44	121.7	
SA46	69.4	
SA47	121.0	
SA48	68.2	
SA49	64.3	
SA51	117.2	
SA55	93.9	
SHL91	33.8	
S7841	111.3	28.1
S11274	40.8	23.1
S11275	137.3	
S11282	40.2	
Median	82.2	25.8

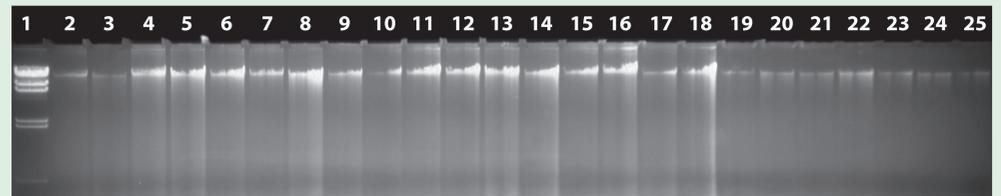
- A 500 µL aliquot of a nasal sample collected from sheep using Performagene and purified using PG-L2P gave a median concentration of 82.2 ng/µL and a minimum concentration of 33.8 ng/µL.
- The median amount of DNA collected using the nasal swab was 8.2 µg. This is an amount of DNA well in excess of requirements for the OvineSNP50 BeadChip.

Figure 2: Representative image of visual QC check



- Nine of the nasal samples did not pass the visual QC test due to a distinct yellow colour.
- Regardless of the sample colour all samples were subsequently analyzed using the OvineSNP50 BeadChip.
- Visual QC is a subjective assessment of quality. The colour observed in nasal samples does not appear to impact performance on the OvineSNP50 BeadChip.
- Further purification of the samples beyond alcohol precipitation can be used to eliminate the material contributing to coloured samples.

Figure 3: High molecular weight QC agarose gel of nasal and blood DNA samples



- 2 µL of each sample was run on an agarose gel to evaluate presence of high molecular weight DNA, with lanes 2-19 corresponding to nasal samples and lanes 20-25 corresponding to blood samples.
- All samples were observed to contain high molecular weight DNA.
- As a constant volume was loaded on the gel, some lanes appear overloaded while some are under loaded. This is a reflection of the concentration differences between blood and nasal samples.

Table 2: Summary of results from Illumina OvineSNP50 BeadChip

Animal ID	Nasal sample call rate	Blood sample call rate	Saliva/blood concordance
SA6	99.89%	99.87%	100.00%
SA34	99.73%	99.58%	100.00%
SA36	99.29%		
SA38	99.89%		
SA39	99.90%		
SA42	99.93%		
SA44	99.92%		
SA46	99.75%		
SA47	98.63%		
SA48	99.69%		
SA49	98.63%		
SA51	99.89%		
SA55	99.88%		
SHL91	44.25%	99.91%	
S7841	99.39%	99.76%	100.00%
S11274	99.84%	99.02%	100.00%
S11275	99.56%		
S11282	99.80%	99.63%	100.00%
Median	99.80%	99.70%	100.00%

* sample SHL91 was removed from the median calculation due to sample or chip

- The median call rate for gDNA from nasal samples was 99.8%.
- One of the 18 nasal samples did not perform well on the OvineSNP50 BeadChip despite having sufficient DNA that was assessed to be of high molecular weight. At this time it remains unclear if the failure was due to sample or procedure or BeadChip. Further work including re-purification and re-running on the OvineSNP50 BeadChip will be performed to evaluate the issue.
- The median call rate for gDNA from blood samples was 99.7%.
- The genotype concordance for paired nasal and blood samples was 100%.

Conclusions

- Performagene provides a quick and easy method for collecting large amounts of high quality DNA from sheep using non-invasive methods.
- DNA collected using Performagene can be used on the OvineSNP50 BeadChip.
- DNA collected from nasal samples is the same as DNA from blood as shown through the 100% genotyping concordance rates.